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Central College of Agriculture
Laboratory of Applied Entomology
Warsaw, Nakowiecka 8

Jan Poczek

Extra

STUDIES ON NUTRITIONAL REQUIREMENTS OF SELECTED SPECIES OF [illegible] OF IMPORTANCE TO STORED PRODUCTS

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S u m m a r y

The subject of this work is to determine food components required by mites- pests of stored food products. Mites are small animals, measuring in average 0,5mm. They are relatives of spiders, living very often in all kind of foods. They propagate in the products very quickly, eat them and contaminate with their feces and moulting-skins. Many species, including the species discussed in the present work are distributed over the world and cause many troubles in stores everywhere.

The influence of various diets containing simple components like proteins, carbohydrates, vitamins, mineral salts was studied on three very common species. Working on bigger animals physiologists usually control the effect of artificial diets by weighing the animals. In this case it was impossible and therefore: (1) the time of development, (2)mortality during development, (3)longevity of adult mites and (4)fecundity of females of mites reared on these artificial diets in special rearing cages were studied. All cultures were kept in temperature 20°C and in 80% of relative air humidity. The data received were compared to the similar ones obtained in cultures of the mites reared on beer yeast and wheat germ as check-foods. The longevity have been compared to the longevity of mites kept in empty cages.

23 various artificial diets were examined during the report period. The main point of the introductory work was the determination of the suitability of various proteins and the finding of basic food requirements of the animals. No data have been known on this subject in the literature.

It was found till now that for one species gelatin, for second species casein, gluthen or gelatin and for third casein gluthen or albumin were the best proteins. All the three species need in the artificial diet, besides of proteins, energetic substances like carbohydrates and fats as well as complex of vitamins and of mineral salts. Some ingredients like cholesterol which are necessary in the food of all insects are not needed in the food of the mites although they live in same habitats as many insects.

Out of the four above mentioned biological values the fecundity of females showed the greatest dependability upon food. However, time of development, mortality during development and longevity varied distinctly (even received on relatively good diets) in comparison to check-foods. It indicates that we are still quite far from understanding the food requirements of the mites.

The explanation of the problem would deepen the knowledge on this interesting and economically important group of animals. We want to study further the effect of various foods on the physiological state of mites and to look for some relations between this state and the sensitivity to various chemicals applied in the control of mites. Many new biological data will be also collected during these studies.

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Introduction

One of the greatest troubles connected with the storage of foods in many parts of the world especially with humid climate is the infestation by mites. There are known about 30 species of mites of families Acaridae and Glycyphagidae attacking various products. Although many of them are polyphagous some preferences of some species were already found. The study of the habitats and the biology of mites may help in figuring of the origin of infestation and in elaborating methods for prevention and control.

There are very few data dealing with physiology of these mites. Determination of nutritional requirements of at least some of these species will enable better understanding of differences between them, pertaining to the variability of their habitats, processes of their feeding as well as the infestation.

Three species of mites were chosen for these investigations. (1) Carpoglyphus lactis (L.) a very common pest of dried fruits occuring almost exclusively in storages and houses. (2)Tyrophagus infestans (Guds.), known as living on plants and more rarely in stored products, and (3) Acarus siro L., a very common species occuring in soil and organic remnants under field conditions and a very common pest of all kind of stored products.

Sakhvatkin (1941) and Vitzthum (1943) found C.lactis on dried fruits and products containing lactic, acetic or succinic acid. Honeycombs, rotting vegetables, fermenting drinks like wine, beer, fruit juice are also very often infested by this species. Hughes (1960) stated that this species prefers food substances containing sugars in which bacterial or fungal activity give rise to the lower fatty acids. In species of this genus there is a tendency to feed on materials which are or were subjected to bacterial or fungal activity. They attack both dried or liquid products but mostly in stores and only exceptionally under field conditions.

There are no data concerning the feeding of T.infestans. Hughes (1961) used to find this species in pastures, in chaff, on many plants under field conditions. According to my previous investigation results (Boczek et al., 1960) species belonging to the Tyrophagus genus occur more frequently on foods containing large amounts of fats and proteins e.g.seeds of oil plants. Arnecskowski (1961) was looking for food preference of Tyrophagus noxius n.z. and found that the species attacks most willingly dried mushrooms then Swiss cheese, wheat germs and hemp seeds. Products like powdered milk, broken rice seeds, potato starch were not attractive for this species. Hivard (1958) observed that populations of Tyrophagus castellani (Hirst) reared on cereal flakes and yeast fed on mycelium of moulds, mostly Aspergillus, rather on food material. He got good results later (Hivard, 1959) rearing the mites on moulds. According to Andre (1933) mites of Tyrophagus genus may be responsible for heavy losses during the storage of tobacco. They are serious laboratory pests of insects and fungus cultures

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(Jewson & Tattersfield, 1922; Sage & Shafik, 1956; Norris, 1946). They interfere with the cultivation of mushrooms (Jary & Stapley, 1957; Davis, 1944). Some species of Tyrophagus genus were found in field (Griffiths, 1960; Boczek, 1961).

Flour mite is found as pest on all kinds of products like cheese, grain, hay, linseed, barley, in disused beehives and on the surface of flour (Hughes, 1961). According to Boczek (1957) the best food for the species are consecutively: dried meat, yellow cheese, powdered milk, grain germs, flour, seeds of oil plants. Only seeds with damaged coat are attacked. Many authors observed this species under field conditions, in plant remnants on the surface or in the soil (Sorokin, 1951; Griffiths, 1960; Boczek, 1961). Several authors (Romanova, 1936; Scionov, 1940; Solomon, 1940) stated that mites eat in attacked grain only germ and aleuron layer. Only rarely endosperm may be attacked. According to Sigrianski (1940) grain stored under conditions of high relative humidity is overcome by moulds and then starch is decomposed to simple sugars which can be then digested by mites. Acarus siro can develop, similarly like previous species, on moulds (Boczek, 1957; Sozynski et al., 1958). Klodecki (1959) studied the changes caused by mites in infested flour. He found an increase of nitrogen compounds as an effect of great losses in carbohydrates. Polypeptides and free aminoacids are formed by mites in process of digestion of proteins consumed. Nitric compounds, mostly guanine, are excreted. Only about 10% of nitrogen compounds is used by mites for building of their tissues.

But was quoted above and what is protable the whole material concerning the food requirements of these three species it can be stated that we have till now very few informations in the field. Much more is known about the foods of stored product insect pests. Most works were done with Blatta, Tribolium and Tenebrio molitor, which live frequently with mites. Especially worth to mention are papers of Fraenkel and his associates of Urbana, Illinois. Accordingly to Fraenkel's paper of 1950 which sumes up some previous results obtained with Tenebrio the insect can grow and propagate in food containing 80-85% of carbohydrates. The effect of glucose and starch is similar. Casein, lactalbumin are the best proteins. Cholesterol is a necessary ingredient for this and almost for all other insects (Hoefer, 1953). Addition of 5% yeast makes flour an optimal food. Many B-vitamins are also necessary in food of this species. There is no indication that fat is required. Luckey (1954) gives a prescription of an universal diet for all living organisms.

1. Experimental procedures

All cultures of mites have been conducted in special rearing cages which make possible to repeat observations even very frequently without irritating the animals. The cages were previously described by the principal investigator (Boczek, 1954). The cages have been kept in dessicators with saturated solutions of NH_4Cl giving relative humidity of 80%. Dessicators have been kept in constant temperature chambers with temperature of 20°C. The mites are reared on artificial diets prepared by the biochemist and thoroughly mixed. Small quantity of food is given

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Table 1.

Composition of artificial diets (ingredients in per cents)

ingredients \ diets	A	B_0	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8	B_9	B_{10}	B_k	C	Z	F	G	E	I	J	K	L	M
Agar-agar	-	4	4	4	4	4	4	4	4	4	4	4	-	-	-	-	-	-	3	3	6	6	6
albumin	90	-	-	-	-	25	-	-	25	25	-	-	-	-	-	-	22,5	-	6	-	-	-	-
casein	-	-	25	-	-	-	-	-	-	-	-	-	15	90	-	-	22,5	30	6	8	19	-	-
gelatin	-	-	-	-	25	-	-	25	-	-	-	25	-	-	90	-	22,5	30	6	8	-	-	-
glucose	-	15	15	15	15	15	15	15	15	20	20	20	70	-	-	-	-	-	13	13	25	25	25
gluthen	-	-	-	25	-	-	25	-	-	-	25	-	-	-	-	90	22,5	30	6	8	-	19	18
l-lisin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0,5
mineral salts	-	5	5	5	5	5	5	5	5	5	5	5	4	-	-	-	-	-	5	5	8	8	8
potato starch	-	30	30	30	30	30	29	29	29	25	25	25	-	-	-	-	-	-	27	27	-	-	-
sacharose	-	40	15	15	15	15	15	15	15	14	14	14	-	-	-	-	-	-	14	14	25	25	25
soybeam oil	-	5	5	5	5	5	5	5	5	5	5	5	-	-	-	-	-	-	5	5	7	7	7
vitamins	10	1	1	1	1	1	1	1	1	1	1	1	10	10	10	10	10	10	9	9	10	10	10
cholesterol	-	-	-	-	-	-	1	1	1	1	1	1	$	-	-	-	-	-	-	-	-	-	-

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Table 1. Data obtained with different foods

Food	Mean time of development of one generation, in days	Mortality during development, in per cent	Mean (and maximum) longevity, in days	Mean (and maximum) fecundity, of single female
T. infestans (male)				
without food				
[illegible]	[illegible]	100	4(8)	0
wheat germ	27	57	72(154)	133(215)
b_0	[illegible]	100	37(40)	0
b_1	[illegible]	89	57(113)	21(41)
b_2	27	56	56(119)	11(27)
b_3	42	77	67(144)	60(175)
b_4	[illegible]	100	32(40)	0
b_5	[illegible]	100	30(38)	0
b_6	[illegible]	100	30(41)	0
b_7	[illegible]	100	32(40)	0
b_8	[illegible]	100	27(40)	0
b_9	[illegible]	100	29(32)	0
b_{10}	43	86	32(47)	37(52)
b_k	[illegible]	100	25(72)	0
[illegible]	39	70	x	x
[illegible]	38	90	x	x
T. sordida (male)				
without food				
[illegible]	[illegible]	100	22(63)	0
yeast	19	26	53(128)	60(156)
b_0	0	100	37(87)	0
b_1	[illegible]	100	41(64)	0
b_2	[illegible]	100	25(51)	0
b_3	31	48	43(106)	12(37)
b_4	[illegible]	100	38(51)	0
b_5	[illegible]	100	46(76)	0
b_6	30	96	46(100)	18(34)
b_7	[illegible]	100	12(47)	0
b_8	[illegible]	100	40(73)	0
b_9	[illegible]	100	49(91)	0
b_{10}	[illegible]	100	54(85)	0
b_k	15	98	37(62)	0
R. sanguineus (male)				
without food				
[illegible]	0	100	7(28)	0
wheat germ	16	30	53(85)	**132(424)**
b_0	0	100	10(24)	**0**
B_1	26	74	30(81)	**52(187)**
B2	38	[illegible]	28(48)	**10(43)**
B_3	0	100	19(42)	**0**
B4	0	100	[illegible](33)	0
b_5	0	100	47(57)	22(82)
B6	0	100	22(40)	0
B7	34	94	35(53)	6(29)
B_8	22	[illegible]	25(54)	6(13)
B_9	29	[illegible]	30(90)	21(71)
B10	0	100	17[illegible]	0
Bk	20	[illegible]	42[illegible]	70(90)

(x) = the experiment is not finished yet

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on the bottom of cages and then mites are added. As check-food yeast for C.lactis, wheat germ for A.siro and wheat germ and yeast for T.infestans are applied. The mites are very small animals and they can not be measured and weighed. The suitability of artificial diet is therefore checked by the comparison to the data received with check-foods of four following values: (1) mean time of development of one generation, in days; (2) mortality during development in percents; (3) mean (and maximum) longevity in days; (4) mean(and maximum) fecundity of one female. Each experiment is conducted this way: 50 eggs laid on given day are put to the cage with food examined and then in every other day observations the data mentioned under (1) and (2) are gathered. Then the longevity of 25 specimens kept in separate cages, with known birth-day, which did not copulated and laid eggs is observed. Lastly the fecundity of 25 females kept with males in separate cages is counted by removing eggs every third day. The longevity is additionally controlled by comparison with the longevity of specimens kept in cages without food.

Some investigators (e.g.Rivard, 1956 & 1959) used to conduct similar experiments with 15 replicates but we find the number too small. There are very great differences in each experiment and therefore the error would be too big. Because of small size of the animals the establishment of experiments and carrying out of observations is very time-consuming and must be done by trained people.

2. Results and discussion

During the first 11 months of the investigation rearings of three species of mites on check-foods, without food and on 23 artificial diets were carried out. Detailed investigations were made for C.lactis and A.siro on 12 and for T.infestans on 14 diets. The composition of all 23 artificial diets is presented on table 1 and all detailed biological data of studies - on table 2.

Observations indicate that no one of the artificial diets applied results in growth and development of species comparable to those fed with check-food.

The best for the development of T.infestans were diets b_1, I and J. Diet b_1 contains one protein- casein, energetic substances, mineral salts and vitamins. Diets I and J have the mixture of various proteins. The fecundity of the species was the highest on diet b_3 containing only one protein- gelatin, energetic substances, mineral salts and vitamins. The development of the species was observed on all of 6 diets (b_1, b_2, b_3, b_{10}, I, J) and on all of them eggs were laid by females but longevity was usually lower (even on diet b_1 & b_2) than on check-foods. The mortality on diet b_2 was significantly lower than on yeast and comparable to the mortality on wheat germ. The longevity of mites kept on artificial diets was always longer than of mites kept in rearing cages without food.

Growth and development of mites was not observed on diet without any protein (b_0); on diet with albumin as single protein (b_4); on diet with only 1% of casein as single protein

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(B_k); on diets without mineral salts (A, C, E, F, J, H); on diet without energetic substances even in cases when 90% of the diet was in form of protein (A, C, E, F), or even if various proteins were mixed in the diet (G, H). Hardly understandable is the lack of development on diets B_5, B_6 and B_9 which are diets B_2 and B_3 with the addition of cholesterol or cholesterol and glucose. Development on diets without cholesterol indicates that this ingredient is not needed for the species. However it can be assumed with very low probability that cholesterol has the inhibiting effect. On diet B_{10} with cholesterol the development was observed. Some unexpected finging is the fact that the development of mites was observed on diets B_3 and B_{10} containing gelatin. This compound contains aminoacids without tryptophan - a very aminoacid important for many animals.

Even on good diet B_1 the development of one generation took 9-11 days longer than on check-foods. Much greater differences were stated in fecundity. Mean fecundity on diet B_1 was 6-10 times lower than on check-foods, on diet B_3 it was more than twice lower.

Data dealing with Carpoglyphus lactis differ distinctly from those of previous species. The development of species took place only on artificial diets B_3 and B_6. On diet B_k only one specimen out of 50 eggs grew up and no one egg laid by female on this food was found. It is interesting that in this case time of development of one generation was the same as that on yeast i.e. 15 days. The mortality during development was the lowest on diet B_3. The longevity was in general very variable and it is difficult to establish any regularity. On diet B_7 the longevity was lower than in rearing cages without any food. The highest longevity, even longer than on yeast was observed on diet B_{10} although no development and no eggs were found. On both diets B_3 and B_6 only relatively few eggs were laid.

Diets B_3 and B_6 have almost the same composition, B_6 has only one additional component - cholesterol. Including B_k we can say that for the development of the species gelatin is the best protein, much worse is casein. Mass cultures kept on next artificial diets showed that the best ones will be probably diets I & M having another protein - gluthen. Anyhow, for the development of the species besides of protein energetic substances, mineral salts and vitamins are necessary. The growth of mites was not observed on diets having albumin as single protein (B_4, B_7, B_8); on diets lacking energetic substances and mineral salts (C, E, F) even if the mixture of various proteins was used (G, H). The development on diet B_3 without cholesterol shows that this ingredient is not needed for the species.

As regards Acarus siro the results received were still different. The development was observed on 6 out of 12 investigated artificial diets. The shortest was the development of one generation on diet B_k. On this food the mortality during development was the lowest and the mean longevity and fecundity were the highest of all. It seems that diet B_k was the best. However, even in this case the mortality was much higher than

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on wheat germ. The longevity of mites was always few times higher on artificial diets than of mites living without any food. The lowest was the mortality on diets lacking protein or containing gelatin as single protein (B_0, B_3, B_6, B_{10}). On diets on which the development was observed the longevity was 4-5 times higher than without food. On diet B_5 eggs were laid by females although the mortality during the development was 100% and no adults grew up.

Comparison of the composition of diets which enable the development of Acarus siro leads to the following considerations. The development took place on diet B_2 and B_9 containing gluthen but diet B_5, where development was not observed is very similar (plus cholesterol) and it is hardly to be suggested that cholesterol made the development impossible. B_1 and B_4 diets contain casein and it seems that this protein would be very essential and suitable component for the species. On diets I and J having also casein some eggs were observed and development went very slowly. It is quite possible that the low amount of the component innibited the development. Good results were received also with albumin present as single protein (B_7 and B_8). Fourth protein tested - gelatin was not suitable for the flour mite. Looking at artificial diets on which development did not occur we find that like in previous two species in good artificial diet protein with energetic substances, vitamins and mineral salts have to be mixed.

Some results of the preliminary studies should be still supported by additional replicas. Sometimes in our first experiments better results were obtained keeping mites on greater amounts of diet. We found while the mixture was not exactly mixed. Complexes of vitamins and proteins therefore were made at first, later this diet was dissolved in water, dried and powdered. In many cases diets gave better results after 1-2 months of laying in refrigerator than that freshly prepared.

Much better would be to use instead of proteins - mixtures of aminoacids. Both proteins and aminoacids should be absolutely clean. Very small amounts of other substances can change wholly the results. All these compounds available in Poland do not give full garantee. Hence we would appreciate very much receiving some small amounts of compounds of which list is included to the first copy of the report.

5. Conclusions

The best results with T.infestans were received on foods containing one of three proteins: casein, gluthen or gelatin mixed with energetic substances: glucose, staren, sacharose, and soybean oil or with diets containing the mixture of proteins with energetic substances. Mineral salts and vitamins are also needed. Luckey's prescription of vitamins gave good results. Cholesterol is not needed in the food.

The best for C.lactis is the diet containing gelatin, much worse is probably gluthen and casein. To the proteins energetic

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substances have to be added. Without these various compounds the mortality is very high and females do not lay eggs. Mineral salts (e.g.ash of milk) and vitamins (Luckey's mixture) are needed too. The development of the species can take place on dry foods kept in 80% of relative humidity. Cholesterol does not play any role in diet for the species.

From all 23 artificial diets tested till now for A.siro about 10 were promising and from 12 studied in details 6 were suitable. Good food for the species should have one of three proteins: casein, gluthen or albumin or their mixture. To the protein energetic substances, carbohydrates and fat must be added. Better results were received with the mixture of simple sugars than with starch. Mineral salts and vitamins are also needed. Cholesterol does not show any effect on the development.

The differences showed till now between the three studied species of mites deal only with protein requirements. Lack of cholesterol in diet does not inhibit the development, hence the mites differ from insects. Out of four biological values taken into consideration the fecundity of females is probably the most influenced by kind of food. Here the differences between check and artificial foods were always the greatest.

4. Plan for future work

During the coming year we are planing to look further for diets on which the development would go as similarly as possible with that on check-foods. We want also replace proteins by aminoacids. Various carbohydrates will be also checked as components of diets. We are going to try to analyse the homogenates of mites reared on different foods - more or less suitable for their growth and development. First of all the amounts of such components like general nitrogen, fats and mineral salts will be determined.

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